The membrane potential can alter the local membrane structure as a result of the response of a charged or zwitterionic lipid to the local electric field. We have investigated the variation of lipid packing in response to interfacial electric potential differences across the monolayer that vary from −0.12 V to +0.28 V. Controlled variation of the potential difference was imposed on an SOPC (1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine) monolayer at 1,2-dichloroethane/water interfaces by the use of a 4-electrode liquid/liquid electrochemical cell. X-ray reflectivity and interfacial tension measurements reveal that the lipid layer is essentially unchanged until the potential reaches 0.18 V, at which point the lipid layer thickens decreases and the area per lipid increases. These data are qualitatively consistent with the expected variation of the lipid layer due to the torque on the headgroup dipole moment. Molecular dynamics simulations confirm the angular variation of the headgroup with electric potential difference. Electron density profiles determined by the simulations show the same trends as the x-ray reflectivity data.

944-Pos Board B713

Happy Marriages among Biophysical Techniques

Kunchithapadam Swaminathan.
National University of Singapore, Singapore, Singapore.

Even though trained as an X-ray crystallographer, the author shares his experience on why and how he learned and engaged other biophysical techniques.

The regulatory (R) subunit of protein kinase A (PKA) modulates the activity of PKA during translation of extracellular signals into a biological response in a cAMP-dependent manner. PKA exists in two distinct and structurally dissimilar conformations. In our work, we engaged X-ray crystallography, structural proteomics approaches, amide hydrogen/deuterium exchange and ion mobility mass spectrometry[1].

Hibiscus latent Singapore virus (HLSV) is a rigid rod-shaped plant virus and a new member of the Tobamovirus family. Unlike all other Tobamoviruses, the HLSV genome contains a unique poly(A) tract in its 3′ untranslated region which plays a crusial role in viral infection. We determined the structure of HLSV by X-ray fiber diffraction. The nucleotide recognition mechanism of HLSV during virus assembly is discussed[2].

Thioredoxins (Trxs) play a key role in maintaining a redox environment in the cell. They act as potential reducing agents and are also known to activate the DNA binding activity of NF-κB, an important transcription factor. In our work on 16-kDa Trx-like protein from *Carcinoscorpius rotundicauda* (CrTRP16), we solved the structure by NMR and explored the molecular basis of NF-κB activation using NMR, analytical ultracentrifugation and other techniques[3].

1. Badireddy, S. et al. (2011). Mol. Cell. Proteomics, 10:M110.004390.
2. Tewary, S.K. et al. (2011). J. Mol. Biol., 406, 516-526.
3. Giri, P.K. et al. (2012). J. Biol. Chem, 287, 29417-29428.

945-Pos Board B714

Getting Better Information into and Out of High-Resolution Structures

Lindsay N. Deis.
Duke University, Durham, NC, USA.

My research aims to aid some of the difficult but important parts of model building at very high resolution. Using ubiquitin as a test case, I am working to improve the modeling of alternate conformations both at room temperature and at cryogenic temperature. In addition, we hope to advance the modeling of ions, in particular the relationship of partial occupancy ions to alternate conformations. I currently have three high-resolution structures at cryo-temperature between 1.0Å and 1.2Å, and I have room-temperature structures between 1.3Å and 1.5Å. We are currently working to identify alternate networks for mutagenesis and further structural studies. By collecting high-resolution data in a variety of space groups and conditions, we hope to better understand the link between ubiquitin conformational variability and binding specificity.

Another aspect of my project involves getting better information into high-resolution models. Our group has previously developed all-atom contact analysis and the *MolProbity* web service, and these features are now incorporated into the *PHENIX* crystallographic software system. However, our tools position hydrogens at the nucleus, but *PHENIX* places them at the centers of the electron clouds. Because the electron cloud is what diffracts X-rays and what matters in evaluating steric contacts, we are working to update parameters in both services. In conjunction with collaborators who are assessing the hydrogen bond-lengths, my work uses a database-drive approach to evaluate our current van der Waals radii. Recalibrating the van der Waals radii is important because the radii directly influence contact analysis. We have now settled on a new set of bondlengths and radii and are working to implement these values in both *PHENIX* and *MolProbity*. Our new parameters will improve the validation tools available to the crystallographic community, thereby allowing our users to better detect model errors.

946-Pos Board B715

A Micro-Beam X-Ray Scattering Beamline Dedicated to Life Science Research at NSLS-II

Lin Yang.
Brookhaven National Laboratory, Upton, NY, USA.

We report the progress in the development of the High Brightness X-ray Scattering for Life Sciences (LiX) beamline at NSLS-II, a new synchrotron source that is currently under construction at Brookhaven National Laboratory. The LiX beamline is part of the Advanced Beamlines for Biological Investigations using X-rays (ABBIX) project funded by National Institute of Health. It will provide state-of-art capabilities in 3 scientific areas: time-resolved biomolecular solution scattering based on microfluidic flow cells, diffraction from single- and multi-layered lipid membranes, and scattering-based scanning probe imaging and tomography of biological tissues. The final design of the LiX beamline is currently underway, with the start of operations scheduled in 2015.

947-Pos Board B716

Macchess: Unique Opportunities for Structural Biology at a Synchrotron Source

Chae Un Kim, Richard Cerione, Michael Cook, Richard Gillilan, Sol Gruner, Qingqiu Huang, Irina Kriksunov, William Miller, David Schuller, Scott Smith, Doletha Szebenyi.
MacCHESS, Cornell University, Ithaca, NY, USA.

MacCHESS (“**Mac**romolecular diffraction at **CHESS**”) is an NIH funded facility at the Cornell High Energy Synchrotron Source; we provide a user facility with exceptional staff support. A special MacCHESS strength is developing new x-ray methods to benefit the entire structural biology community.

Crystallography - High-flux monochromatic beamlines equipped with large CCD detectors, air bearing-based goniometers, selection of 100-μm or 20-μm beam size, cryocooling, excellent crystal centering systems, and crystal automounters are available. BSL-2 biohazards can be handled. Methods for collecting and processing multi-crystal data sets are under development.

BioSAXS - A dedicated beamline features a dual SAXS/WAXS setup using 2 Pilatus detectors and an integrated computer-controlled flow system including robotic sample loading from 96-well trays, a capillary cell with a replaceable insert, and a convenient graphical interface. An HPLC system allows sample preparation immediately before taking data. Microfluidic “lab-on-a-chip” units are under development. Periodic workshops are held to educate users in the intricacies of BioSAXS.

Pressure cryocooling - A new method for cryocooling crystals under pressure reduces both cooling-induced degradation and the need for penetrating cryoprotectants, and can stabilize mobile ligands and possibly reaction intermediates. We offer pressure-cooling as a service to CHESS users, while continuing to develop the method. Several sample mounting techniques are now available, and the technique has promise for use with biological samples other than crystals.

More information - To request beamtime, fill out a simple on-line proposal form at http://www.chess.cornell.edu. Mail-in service is available for crystallography. We welcome a chance to collaborate on “non-standard” experiments. For more information, contact User Administrator Kathy Dedrick (kd73@cornell.edu).